

## NIH Guidelines - Biosafety Level 3 (BL3)

These questions are based on the Biosafety Level 3 (BL3) section of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, March 1996 edition, Appendix G-I and G-II-C.

Please circle the response that best describes the laboratory in which work with select agents will be carried out.

**N.A. = not applicable. If you mark "N.A." , please provide a brief explanation below that item or on a separate page.**

### Appendix G-I. Standard Practices and Training

Yes, No, N.A.      The first principle of containment is strict adherence to good microbiological practices. Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction. At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Yes, No, N.A.      Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided.

### Appendix G-II-C-1. Standard Microbiological Practices (BL3)

Yes, No, N.A.      Appendix G-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Yes, No, N.A.      Appendix G-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Yes, No, N.A.      Appendix G-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Yes, No, N.A.      Appendix G-II-C-1-d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Yes, No, N.A.      Appendix G-II-C-1-e. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Yes, No, N.A.      Appendix G-II-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Yes, No, N.A.      Appendix G-II-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Yes, No, N.A.      Appendix G-II-C-1-h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

### Appendix G-II-C-2. Special Practices (BL3)

- Yes, No, N.A. Appendix G-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.
- Yes, No, N.A. Appendix G-II-C-2-b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.
- Yes, No, N.A. Appendix G-II-C-2-c. The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
- Yes, No, N.A. Appendix G-II-C-2-d. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.
- Yes, No, N.A. Appendix G-II-C-2-e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.
- Yes, No, N.A. Appendix G-II-C-2-f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.
- Yes, No, N.A. Appendix G-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.
- Yes, No, N.A. Appendix G-II-C-2-h. An insect and rodent program is in effect.
- Yes, No, N.A. Appendix G-II-C-2-i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.
- Yes, No, N.A. Appendix G-II-C-2-j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
- Yes, No, N.A. Appendix G-II-C-2-k. Molded surgical masks or respirators are worn in rooms containing experimental animals.
- Yes, No, N.A. Appendix G-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.
- Yes, No, N.A. Appendix G-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units, open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

- Yes, No, N.A. Appendix G-II-C-2-n. All wastes from laboratories and animal rooms are appropriately

decontaminated before disposal.

- Yes, No, N.A. Appendix G-II-C-2-o. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.
- Yes, No, N.A. Appendix G-II-C-2-p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
- Yes, No, N.A. Appendix G-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the facility Safety Office. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.
- Yes, No, N.A. Appendix G-II-C-2-r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.
- Yes, No, N.A. Appendix G-II-C-2-s. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.
- Yes, No, N.A. Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3). Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment.

#### Appendix G-II-C-3. Containment Equipment (BL3)

- Yes, No, N.A. Appendix G-II-C-3-a. Biological safety cabinets (Class I, II, or III), or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

#### Appendix G-II-C-4. Laboratory Facilities (BL3)

- Yes, No, N.A. Appendix G-II-C-4-a. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.
- Yes, No, N.A. Appendix G-II-C-4-b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

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| Yes, No, N.A. | Appendix G-II-C-4-c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.   |
| Yes, No, N.A. | Appendix G-II-C-4-d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.  |
| Yes, No, N.A. | Appendix G-II-C-4-e. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.   |
| Yes, No, N.A. | Appendix G-II-C-4-f. Windows in the laboratory are closed and sealed.   |
| Yes, No, N.A. | Appendix G-II-C-4-g. Access doors to the laboratory or containment module are self-closing.   |
| Yes, No, N.A. | Appendix G-II-C-4-h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.  |
| Yes, No, N.A. | Appendix G-II-C-4-i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.   |
| Yes, No, N.A. | Appendix G-II-C-4-j. The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system. |